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Title: CARDIOVASCULAR PROSTHESES WITH A STABLE ENDOTHELIAL CELL SURFACE

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Cardiovascular prostheses with a stable endothelial cell surface

Description

The invention relates to cardiovascular prostheses with a stable, confluent endothelial cell surface which is produced by proliferation under a shear stress. Said cardiovascular prostheses are produced by means of a novel method for forming a stable confluent endothelial cell monolayer. Under the term "cardiovascular prostheses", vascular and heart valve prostheses are understood which are covered with endothelial cells on the blood contact side.

Methods for a blood contact side lining of cardiovascular prostheses with endothelial cells are known, even those, wherein a confluent endothelial cell monolayer is obtained by growth of the cells ensuing under static conditions, after an initial sub-confluent seeding or by means of a direct confluent settlement of the isolated cells (US-Patent 5,334,879). Thereby, the seeding may be carried out under a stepwise and/or permanent rotation, and also statically.

Implants from cell structures can also be produced by means of an absorbable carrier structure subsequent to enveloping the cells (DE 43 06 661), as a 3-dimensional, as well as a 2-dimensional implant, whereby carrier materials of different manufacture are possible (WO 94/17841). In the document EP 0 320 441, a method is described for conditioning plastic carriers covered with living cells – the adaptation to a shear stress is supposed to be obtained. Thereby, a periodically interrupted medium current is used – a permanent shear stress is not applied. In this case, it is the conditioning of the carrier covered confluently with cells and a habituation of the deposited cells to shear stress which is concerned, and not an adaptation of the cells as early as during their division and adhesion processes.

The application of shear forces is also the subject matter of the document WO 93/01843. According thereto, however, only shear forces of a very small amount and mainly of a radial orientation are applied. The seeding of the cells thereby ensues under static conditions, with a 4-fold rotation of the prosthesis every 20 minutes by about 90 degrees,

and only after this, a permanent shear stress of a very small amount is generated by rotation on a roller (10 revs./min).

The laid-open document WO 94/25584 reports on the formation of a closed cell monolayer which is generated as early as during the seeding. The initial adhesion takes place under static conditions. Subsequent thereto ensues the incubation of the confluently sown monolayer for over 24 hours. Here, the adaptation of the cells to physiological parameters of the shear stress does not ensue during the proliferation, either. US Patent 5,634,879 reports on a method providing sowing of the cells directly on the prosthesis after the isolation, without aiming at obtaining an adaptation of the cells to an increased defined shear stress. The cells are thereby deposited to the inner surface by means of a transversal filtration of the suspension through the prosthesis material. A closed circulation perfusion does not exist here. No proliferation under defined flow conditions ensues either.

The disadvantage of the described inventions consists in that either the proliferation behavior of the cells under static conditions entails a reduced adhesion of the cells on the prosthesis surface due to a modified gen expression, or no conditions are simulated *in vitro* which correspond to those of the *in vivo* situation.

The object of the present invention consists in developing cardiovascular prostheses by means of which the mentioned disadvantages can be countered.

This task was solved in that in cardiovascular prostheses with an endothelial cell surface, the formation of a confluent monolayer ensues subsequent to an initial sub-confluent population of the surface on the blood contact side. This ensues by the cells growing under a permanent influence of defined pulsatile shear forces increasing up to physiological values, by means of streaming the prosthesis surface on the blood contact side along the main axis of the prosthesis in an inner perfusion circuit, and in that a moistening of the outer prosthesis wall ensues in an outer perfusion circuit or in a permeable medium reservoir.

The essence of the invention resides in a combination of known elements (method for forming a confluent monolayer on the surface of cardiovascular prostheses) and novel

elements (adaptation of the endothelial cells to a hemodynamic shear stress locally present in the blood vessel at the implantation location), which mutually influence and result in their entire action in an advantage of use and the desired success, which resides in that the hemodynamic shear stress directly influences the structure and function of the endothelial cells, and therewith causes an influence on the formation of a confluent endothelial cell monolayer as early as during their growth (cell division phase).

Surprisingly, it has been found that during the formation of the confluent endothelial cell monolayer by cell division, the cell population already becomes adapted to wall shear stress such as they can be observed *in vivo*, with the consequence of a stable adhesion of the cells to the prosthesis surface on the blood contact side, which is of decisive importance for the long-term structural and functional efficiency of the endothelial cell monolayer as an interface between prosthesis surface and the flowing blood. Therewith, the formation of a novel prosthesis surface comparable to *in vivo* linings of blood vessels is achieved, and as a result thereof, a significant reduction of the coagulation risk as compared to uncoated prostheses, or prostheses which are not confluently lined with endothelial cells.

Already known methods are characterized by a seeding of the prosthesis surface ensuing initially and under static conditions, which is only interrupted for short periods and discontinuously for exchanging the medium (EP 0 320 441). Further known are methods wherein the adjustment of the adherence of the cells on the prosthesis surface always takes place along with the formation of the immediate confluence (WO 93/01843). Thus, no proliferation of the endothelial cells under a permanent influence of defined pulsatile shear forces ensues (WO 94/25584).

The invention permits *inter alia* an adaptation of the cells to various values of wall shear stress under consideration of the shear forces arising later locally in the implant in dependence of the implant location, as early as before the implantation of the prosthesis in the blood circulation.

The increasing shear forces can be generated by means of a program-controlled pumping device (7). The mathematical value of the increasing shear forces can be selected variably and time-independently. According to an advantageous configuration, the mathematical value and the final value of the shear forces are allowed to be selected freely

and time-variably via a program control corresponding to the physiological conditions of the implant location, and therewith allow for the formation of arterial as well as venous vascular prostheses (1), according to the various wall shear stresses occurring *in vivo*, and also allow for the adaptation to pulsatile flow conditions.

According to a further advantageous configuration, the mathematical value of the occurring shear forces can be adjusted by varying the pumping capacity, as well as by varying the size of the cross-sections of the pumping tubes used or of any other connecting elements outside of the chamber, as well as by the geometrical form and configuration of the very chamber.

According to a further advantageous configuration, the outer perfusion circuit (5') can be operated in co-current or in counter-current to the inner perfusion circuit (5), but can also be operated statically. The two perfusion circuits (5, 5') can also work as a non-closed system; according to a further advantageous configuration, they lead from one medium reservoir (6) into another medium reservoir (6'), in which the medium which has already flowed through the prosthesis is collected. Thereby, the two circuits can also be joined within the chamber (2) after having flowed past the prosthesis surface.

The inner and the outer perfusion circuit can have different reservoirs or one and the same medium reservoir (6, 6'). The ~~prothesis~~ prosthesis can be present in the very medium reservoir, and thereby, the inner and the outer perfusion circuit can be connected with one another.

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The formation of a confluent endothelial cell monolayer following the seeding of the prosthesis surface on the blood contact side, ensues by means of a perfusion circuit, illustrated in Figure 1 and Figure 2. This circuit is comprised of an inner perfusion circuit for streaming past the prosthesis surface on the blood contact side along the main axis of the prosthesis inside of a perfusion chamber, whereby the prosthesis is fixed and aligned in the inner space of the perfusion chamber by means of an adapter, and therewith constitutes itself a part of the inner perfusion circuit, and is further comprised of an outer perfusion circuit for outwardly streaming past the vascular prosthesis within the same perfusion chamber, which comprises, towards the outside, connections to a pumping device for both circuits, as well as to the medium reservoirs which can be exchanged under sterile conditions, and which also have the function of a pressure equation reservoir.

The outer perfusion circuit serves for moistening the outer prosthesis surface so as to prevent it from drying out. The prosthesis material is often of a high porosity, and can be impregnated prior to the seeding of cells, with fibrin or any other adhesion-promoting substances. An optional perfusion prevents possible gradients from forming in the medium composition, as well as of the pH value at and/or through the prosthesis wall. Thus, a gradient-dependent transversal diffusion through the prosthesis material is prevented from arising.

The invention hence relates to cardiovascular prostheses produced by a novel method characterized by the formation of a stable, confluent endothelial cell monolayer on the surface of the prostheses on the blood contact side. Thereby, the initial seeding of the prosthesis surface on the blood contact side, as well as the following growth of the endothelial cells to a confluent monolayer, ensues under the permanent influence of shear stress generated in two stages: initially, in the seeding phase for generating a sub-confluent endothelial cell monolayer by axial rotation of the culture chamber, and subsequently, by streaming the prosthesis surface along the main axis of the prosthesis. Therewith, conditions are created *in vitro* on the prosthesis surface on the blood contact side, which are comparable to those of the *in vivo* situation. As a result therefrom, a confluent endothelial layer having a high quality is formed.

These novel cardiovascular prostheses ensure markedly improved bonding of the cells on the surface of the prosthesis on the blood contact side and hereby enable the monolayer to be maintained even over long periods and under increased shear stress conditions. Hereby, for the first time, a significant reduction of the risk of coagulation is provided as compared to uncoated prostheses which are not confluently lined with endothelial cells, as well as compared to prostheses which have been confluently populated but exhibit an insufficient bonding of the cells on the prosthesis surface on the blood contact side. This method is suitable for covering heart valves as well as vascular prostheses such as they are used in cardiovascular surgery, hence also stents.

The inventive method consists in that, after an initial sub-confluent seeding of the prosthesis surface on the blood contact side, the formation of a confluent monolayer ensues by the cells growing under a permanent influence of defined pulsatile shear forces

increasing up to physiological values, by means of causing a streaming to occur about the prosthesis surface along the main axis of the prosthesis in an inner perfusion circuit, and by moistening the outer prosthesis wall in an outer perfusion circuit or in a permeable medium reservoir.

The invention will be explained in detail by means of examples, however, without being limited to these.

Examples

Example 1:

A poly(tetrafluoroethylene) bypass prosthesis (Fig. 1: 1) having a diameter of 4 mm and a length of 15 cm is fixed to the inner space of the perfusion chamber (Fig. 1: 2), and hence connected to the inner perfusion circuit (Fig. 1: 5) by means of the adapters (Fig. 1: 3, 3') configured as olive, cones with a clamping or expanding device, respectively. The pulsatile flow (0 – 3,500 ml/h of endothelial cell culture medium) is generated by a peristaltic pump which can be freely and time-variably controlled manually or by a software program (Fig. 1: 7). Via tube connectors (Fig. 1: 4, 4'), which are decentrally mounted at the sides of the perfusion chamber, the outer perfusion circuit (Fig. 1: 5') which is perfused in co-current with the inner circulation, is established by means of silicone tubes. The two perfusion circuits (Fig. 1: 5, 5') each dispose of a reservoir (Fig.: 6, 6') containing endothelial cell culture medium and having simultaneously the function of a pressure equation reservoir. The peristaltic pump (Fig. 1: 7) is so controlled by a computer that the shear forces introduced by a pulsatile flow increase in the prosthesis over a period of 10 hours from 0.01 to 2.5 dyn/cm^2. Thereafter, the flow conditions remain constant until the day of the implantation.

Subsequently, the chamber is opened in a sterile environment, and the prosthesis, confluently covered with cells, is ready to be implanted.

Example 2:

A Dacron® bypass prosthesis (Fig. 1: 1) having a diameter of 5 mm and a length of 10 cm is fixed to the inner space of the perfusion chamber (Fig. 1: 2), and hence connected to the inner perfusion circuit (Fig. 1: 5) by means of the adapters (Fig. 1: 3, 3'). The pulsatile flow (0 – 7,000 ml/h of endothelial cell culture medium) is generated by a peristaltic pump which can be freely and time-variably controlled manually or by a software program (Fig. 1: 7). The outer perfusion circuit is filled (Fig. 1: 5') with medium and statically operated. The two perfusion circuits (Fig. 1: 5, 5') dispose of a common reservoir containing endothelial cell culture medium and serving simultaneously as a pressure equation reservoir. Hereby, expandable blood bags made of the materials ethylene vinyl acetate M (EVAM) or polyvinyl chloride (PVC) are used.

The peristaltic pump (Fig. 1: 7) is so controlled by a computer that the shear forces introduced by a pulsatile flow increase in the prosthesis over a period of 24 hours from 0.01 to 5 dyn/cm². Thereafter, the flow conditions remain constant until the day of the implantation.

Subsequently, the chamber is opened in a sterile environment, and the prosthesis, confluently covered with cells, is ready to be implanted.

Example 3:

A poly(tetrafluoroethylene) vascular prosthesis (Fig. 1: 1) having a diameter of 10 mm and a length of 12 cm is fixed in the inner space of the medium reservoir which therewith represents the outer perfusion circuit, by means of an adapter. The pulsatile flow (0 – 7,000 ml/h of endothelial cell culture medium) is generated by a peristaltic pump which can be freely and time-variably controlled manually or by a software program (Fig. 1: 7), whereby the inner diameter of the pump tubes is variable.

The peristaltic pump (Fig. 1: 7) is so controlled by a computer that the shear forces introduced by a pulsatile flow increase in the prosthesis over a period of 24 hours from 0.01 to 5 dyn/cm². Thereafter, the flow conditions remain constant until the day of the implantation.

Subsequently, the chamber is opened in a sterile environment, and the prosthesis, confluently covered with cells, is ready to be implanted.

Example 4:

A poly(tetrafluoroethylene) bypass prosthesis (Fig. 1: 1) having a diameter of 4 mm and a length of 4 cm is fixed to the inner space of the perfusion chamber (Fig. 1: 2), and hence connected to the inner perfusion circuit (Fig. 1: 5) by means of the adapters (Fig. 1: 3, 3') configured as olive, cones with a clamping or expanding device, respectively. The pulsatile flow (0 – 3,500 ml/h of endothelial cell culture medium) is generated by a peristaltic pump which can be freely and time-variably controlled manually or by a software program (Fig. 1: 7). Via tube connectors (Fig. 1: 4, 4'), which are decentrally mounted at the sides of the perfusion chamber, the outer perfusion circuit (Fig. 1: 5') which is perfused in counter-current with the inner circulation, is established by means of silicone tubes. Hereby, the perfusion of one or of both circulation/s ensues in a non-closed system. Thereby, medium flows from one medium reservoir serving as a storage into another medium reservoir serving as a collecting vessel.

The peristaltic pump (Fig. 1: 7) is so controlled by a computer that the shear forces introduced by a pulsatile flow increase in the prosthesis over a period of 10 hours from 0.01 to 2.5 dyn/cm². Thereafter, the flow conditions remain constant until the day of the implantation.

Subsequently, the chamber is opened in a sterile environment, and the prosthesis, confluently covered with cells, is ready to be implanted.

Example 5:

A heart valve prosthesis (Fig. 1: 1) is fixed to the inner space of the perfusion chamber (Fig. 1: 2), and hence connected to the inner perfusion circuit (Fig. 1: 5) by means of an adapter (Fig. 1: 3). The pulsatile flow (0 – 7,000 ml/h of endothelial cell culture medium) is generated by a peristaltic pump which can be freely and time-variably controlled manually or by a software program (Fig. 1: 7). The outer perfusion circuit is perfused (Fig. 1: 5') in co-current. After streaming past the heart valve prosthesis, the two perfusion circuits unite inside of the chamber (2) until leaving same. The two perfusion circuits (Fig. 1: 5, 5') dispose of a common reservoir containing endothelial cell culture medium and serving simultaneously as a pressure equation reservoir. Hereby, expandable

blood bags made of the materials ethylene vinyl acetate M (EVAM) or polyvinyl chloride (PVC) are used.

The peristaltic pump (Fig. 1: 7) is so controlled by a computer that the shear forces introduced by a pulsatile flow increase in the prosthesis over a period of 24 hours from 0.01 to 5 dyn/cm². Thereafter, the flow conditions remain constant until the day of the implantation.

Subsequently, the chamber (2) is opened in a sterile environment, and the prosthesis, confluently covered with cells, is ready to be implanted.

Figures

Figure 1 shows the schematic structure of a closed perfusion system. The chamber (2) constitutes the main element which is connected, via tube connectors (4, 4') to the inner circuit (5) and the outer circuit (5') with the pumping device (7) and the associated medium reservoirs (6, 6'). The inner circuit is closed by the vascular prosthesis (1) fixed in the chamber by means of the adapters (3, 3'). The arrows shown symbolize the flow direction of the perfusing medium, whereby in the present illustration, a perfusion of the two circuits ensues in co-current.

Figure 2 shows the schematical structure of a perfusion system according to Figure 1, here, however, the perfusion does not take place by means of two closed circuits, but from one medium reservoir (6) into another medium reservoir (6'), in which the medium is collected which has already streamed through the prosthesis.

Reference numerals

1	cardiovascular prosthesis
2	chamber
3, 3'	adapters
4, 4'	tube connector
5	inner circuit
5'	outer circuit
6, 6'	medium reservoir
7	pump